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Tenth Annual Scientific Conference of the Prader-Willi Syndrome Association (USA), July 19, 1995, Seattle, Washington

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INTRODUCTION

Each year for the last 10 years, scientists conducting research on Prader-Willi syndrome have come together to exchange information during a scientific conference held in conjunction with the annual Prader-Willi Syndrome Association (USA) meeting. Presentations based on submitted abstracts encompass such varied fields as genetics, endocrinology, pediatrics, nutrition, psychology, psychiatry, and education. This year's scientific conference was held in Seattle, Washington, on July 19, 1995, in conjunction with the 14th PWSA (USA) meeting held July 20–23. Seventeen reports were presented at the scientific meeting, the abstracts of which follow.

The first part of the session covered reports on genetic research. The multicenter study reported by Nicholls et al. identifies the mechanism responsible for the very small but important percentage of families at risk for recurrence. They described an "imprinting center," which is thought to control maintenance or resetting of imprinting throughout 15q11-q13, and reported patients with mutations leading to abnormalities in the imprinting process. Prenatal diagnosis should be available for these families at risk for recurrence. Another situation permitting prenatal diagnosis is discussed in the work by Kubota et al: trisomy 15 discovered when chorionic villus sampling is done for advanced maternal age. In these cases, molecular analysis on amniocytes will allow for identification of those fetuses with PWS on the basis of maternal uniparental disomy (UPD). Robinson et al. compiled 110 cases of maternal UPD ascertained through PWS and found that most were due to meiosis I errors, associated with advanced maternal age compared to controls. Although most PWS occurs de novo, these findings represent a significant advance in possible prevention.

Two reports compared clinical manifestations in patients with maternal disomy and those with the deletion. Cassidy et al. found that hypopigmentation and the typical facies were more common in the deletion patients. Persistence of feeding problems and earlier onset of hyperphagia in deletion patients were described by Mitchell et al. However, in general, most clinical findings were similar in the two groups.

Atypical patients often contribute important information regarding the mechanism for genetic disorders. Schwartz et al. described an atypical patient with a translocation involving the PWS critical region without an apparent deletion. Further molecular studies of this patient may identify candidate genes for PWS and Angelman syndrome. Dr. Hanchett presented genetic studies by her colleagues, Mowery-Rushton et al., suggesting that mosaicism for the typical 15q deletion detectable by fluorescence in situ hybridization (FISH) may be found in patients with mild or atypical PWS.

Drs. Holm and Dinno presented two patients with clinically atypical Prader-Willi syndrome lacking laboratory confirmation, who appear to have the same disorder, which included hypotonia, obesity, mental retardation, and joint contractures. Several reports described detailed manifestation of PWS. Dr. Hanchett reviewed menstrual history in 106 females seen at the Rehabilitation Institute in Pittsburgh. Among those, almost 50% had spontaneous onset of menses, with onset as late as the thirties and considerable variability in frequency of cycles. Case reports of three children with PWS with critical illness and death in infancy was presented by Drs. Anaya and Clericuzio. They suggested that some infants with PWS may die prior to diagnosis and that factors contributing to an increased incidence of critical illness should be sought. Dr. Bakke et al. studied metabolic rate during rest and exercise and found that whereas the resting rate was mostly normal, the exercise metabolic rate in PWS was slightly decreased when compared to predicted values.

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Two of the reports on behavior characteristics in Prader-Willi syndrome presented some discrepant and some similar findings. Dr. Whitman found major psychiatric illnesses in 12 of 40 persons with Prader-Willi syndrome in Australasia (Australia and New Zealand) and in 19 of 40 in the United States. The psychiatric diagnoses varied, but only 18 of 80 met the diagnostic criteria for neurosis, compulsive type, as defined in DSM-IV. This represents between 20% to 25% of obsessive/compulsive disorder in Prader-Willi syndrome in the age group 2–35 years in her sample. Dykens et al. presented a study on 91 patients, ages 5–47, in whom 60% met the DSM-IV criteria for the same disorder. The reason for the discrepancy was not readily apparent. However, both presenters noted a relationship between family stress and behavioral symptomatology in PWS. In addition, Dr. Whitman reported minimal association between a family history of psychiatric disorders and behavioral difficulties in Prader-Willi syndrome.

An overview of the Vanderbilt University Prader-Willi syndrome program project, recently approved, was presented by Drs. Thompson and Butler. This is a multidimensional project incorporating, among others, genetic, neuroendocrine, nutritional, pharmacological, and psychosocial aspects. Drs. Sulzbacher and Gardner, discussing psychiatric medication in PWS, emphasized that clinical diagnostic criteria of behavioral symptomatology should guide the choice of pharmaceutical intervention. A PWS-specific rating form (PWBRF), which was field-tested at the Seattle parent meeting, has been developed by Dr. Gardner and its use in controlled, double-blind studies in the future was anticipated by the presenters.

The continuing sponsorship of the scientific conference by the PWSA (USA) reflects the organization's awareness that research is of critical importance to its membership. PWS is a complex, multifaceted disorder. This forum has fostered interchange of ideas across disciplines and facilitated collaborative research efforts. PWSA (USA) has applauded the dramatic advances in understanding the genetics of the syndrome during the last few years and is eagerly anticipating future advances also in endocrinology, the behavioral sciences, and other fields of study connected with this disorder. Next year's scientific conference will be held in St. Louis, Missouri, on July 17, 1996.

1

MECHANISMS INVOLVED IN PRADER-WILLI AND ANGELMAN SYNDROME. R.D. Nicholls, J. Amos-Landgraf, A.E. Wandstrat, S. Schwartz, S.B. Cassidy, Y. Ji, D.J. Driscoll. Department of Genetics, Case Western Reserve University; Center for Human Genetics, University Hospitals of Cleveland, OH; University of Florida College of Medicine, Gainesville; Indiana School of Medicine, Indianapolis; Universitäsklinikum Essen, Essen, Germany.

Prader-Willi (PWS) and Angelman (AS) syndromes represent a paradigm for the study of genomic imprinting. Imprinting refers to a differential parent-of-origin specific epigenetic modification of DNA and resulting differential expression during embryogenesis and in the adult. Identification of the genes and mechanisms responsible are necessary to develop therapeutic approaches to these complex genetic disorders. PWS and AS originate most commonly (70–75%) from large de novo deletions of 4 Mb, of paternal or maternal origin, respectively. Maternal uniparental disomy (UPD) for chromosome 15 occurs in 20% of PWS cases and paternal UPD occurs in ~2% of AS patients. Recent studies have identified rare (2–5%) PWS and AS familial cases with an apparent mutation in the imprinting process. The remainder of AS cases have a putative point mutation, consistent with a single gene expressed from the maternal chromosome only, whereas the apparent absence of this class of patients in PWS suggests that at least two genes, paternally expressed only, may be necessary for the classical PWS clinical phenotype.

Deletions in most PWS and AS patients have common breakpoints, and we have identified a low-copy repeat that occurs specifically at the proximal and distal ends of 15q11-q13. This repeat has been characterized by molecular and fluorescence in situ hybridization (FISH) methods in normal individuals and patients with a deletion (PWS or AS), 15q11-q13 duplication or triplication, other rearrangements, and somatic cell hybrids from an individual with a t(15:19) that breaks within the *SNRPN* gene located in the central part of 15q11-q13. This repeat may be responsible for facilitating the common deletion seen in PWS and AS, as well as reciprocal duplications. Furthermore, at least one functional gene is embedded within this complex, low-copy repeat, and it will be important to determine the effect that rearrangements have on this gene(s). These findings may lead to further understanding of the generic and environmental influences in the origin of birth defects.

We first described AS patients with an imprinting mutation, and subsequently we and colleagues have identified and characterized overlapping 6–60 kb microdeletions just upstream of the *SNRPN* gene in multiple PWS and AS imprinting mutation families. These microdeletions define a *cis*-control element that we term the imprinting center (IC), which controls resetting and/or maintenance of imprinting throughout 15q11-q13. IC mutations are hypothesized to arise in the germline (but see below) of one sex, fixing that imprinted state into the chromosome (the "epigenotype"). This mutation can then be transmitted silently through each individual of the same sex at each generation. However, in the opposite sex, IC mutations block resetting of imprinting within 15q11-q13, so that the grandparental epigenotype is inherited. The outcome is inheritance of a disomic epigenotype, resulting in the PWS or AS clinical phenotype. We have identified the de novo origin of the microdeletion in the mothers of two families, and surprisingly, in both cases the event was postzygotic. To date, we have identified a series of imprinted genes in 15q11-q13 that are candidates for phenotypic aspects of PWS (*SNRPN, ZNF127, DN34*), based on expression from the paternal allele only in the human and the mouse, and others have identified similarly expressed transcripts (*IPW PAR-5, PAR-1*). The functional imprinting of these genes, associated DNA methylation imprints, and asynchronous replication are all significantly altered by the IC mutations, suggesting that the IC controls the erasure of and/or resetting the imprinted state (but not maintenance of this state). Significantly, the discovery of IC microdeletions allows prenatal testing in PWS and AS families at significant risk for recurrence.

2

THE ORIGIN OF MATERNAL UNIPARENTAL DISOMY 15. W. P. Robinson, S. Langlois, F. Bernasconi, S. Clark, S. Christian, D.H. Ledbetter, G. Gillessen-Kaesbach, B. Horsthemke, I. Lerer, D. Abeliovich, R. Michaelis, S. Schuffenhauer, A.A. Schinzel. Department of Medical Genetics, University of British Columbia, Vancouver, Canada; Institut für Medizinische Genetik der Univ. Zürich, Switzerland; National Center for Human Genome Research, NIH, Bethesda, MD; Institute für Humangenetik, Universitätsklinikum Essen, Germany; Hadassah Hebrew University Hospital, Jerusalem, Israel; Greenwood Genetic Center, Greenwood, SC; Kinderpoliklinik der Universität München, Germany.

We have presently compiled 110 cases of maternal uniparental disomy (UPD) of chromosome 15 ascertained through the Prader-Willi syndrome (PWS). Investigation of proximal chromosome 15 markers indicates that 77 mat UPD(15) cases were due to meiosis I, 16 to meiosis II, and 17 to somatic errors leading to duplication of the maternal chromosome 15. Overall, a significant reduction in recombination is noted in the MI errors but not the MII errors. This reduction is not simply due to failure to recombine in a proportion of cases as one or more crossover events are normally observed. Examination of maternal UPD cases of MI origin shows significant alteration of recombination in the centromeric, 15q11-15q13 region ($P < 0.02$). Interestingly, recombination is not simply reduced but altered in location, with a deficiency of recombination in a "high" female-specific recombinatory region and an excess of recombination in the vicinity of a male recombination hotspot distal to this site.

A mean age of 34.2 was observed for mother of maternal UPD(15) as compared to 28 in controls. Risk for UPD(15) shows an exponential increase with maternal age similar to that observed for trisomy 21 and may be greater than 1/1000 births for women in their mid-forties. Both maternal

and paternal ages are significantly above the mean for meiosis I and meiosis II errors, but not for cases of somatic origin. There was also no difference in maternal age depending on level of observed recombination. This is in contrast to a recent report that recombination associated with chromosome 21 nondisjunction is decreased in older mothers. The observation of increased maternal age in both meiosis I and II errors further indicates that age effect associated with chromosome 15 nondisjunction is independent of the alteration in recombination.

Cytogenetic results were available in 51 cases including two maternally inherited Robertsonian translocations, two isochromosomes 15, and two small inv dup(15) chromosomes. This frequency (12%) of cytogenetically detectable rearrangements associated with UPD is, however, inflated as two i(15q) and one inv dup(15) cases were analyzed specifically because of these anomalies. These and all other reported i(15q) and inv dup(15q) in association with PWS or Angelman syndrome (AS) have been de novo, whereas all Robertsonian translocations associated with mat UPD(15) have been maternally inherited. By comparison of PWS/AS versus population frequencies of these aberrations, we estimate that the risk of nonhomologous Robertsonian translocation being associated with UPD is ~1/200, whereas the risk associated with de novo small inv dup(15) chromosomes and inherited or de novo isochromosomes is expected to be significantly higher. Interestingly, one mother carried a rob(14;15) chromosome despite a normal karyotype in the PWS-UPD(15) child. Molecular results in the child were consistent with origin from a meiosis II error, but it is conceivable that the abnormality in the mother may have contributed to the abnormal segregation of the homologous chromosome.

3

AN UNUSUAL TRANSLOCATION WITHIN THE PRADER-WILLI/ANGELMAN SYNDROME CRITICAL REGION IN 15q11-15q13 ASSOCIATED WITH CLINICAL PRADER-WILLI SYNDROME. S. Schwartz, T. Grebe, D. Wolff, L. Becker, J. Conroy, R.D. Nicholls, B. Horsthemke, K. Buiting, S.B. Cassidy. Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, OH; Department of Pediatrics, University of Arizona College of Medicine, Tucson; Universitätsklinikum Essen, Essen, Germany.

The absence of normally active paternal genes in 15q11-15q13 as an outcome of either a paternal deletion of that region or by maternal disomy will result in Prader-Willi syndrome. Additionally, in some rare cases, other alterations that usually disrupt the imprinting mechanism of this region are responsible for the syndrome. We report on a patient with Prader-Willi syndrome who has a translocation involving breakage in the Prader-Willi/Angelman syndrome critical region without an apparent deletion.

H.C., a 4-year-old male, had a history of early hypotonia and feeding difficulties. He is currently an obese male (wt >95%) with food seeking behaviors, somewhat thick saliva, and a history of picking at sores. However, he does not have small hands or feet (50–75th%) or small genitalia (10th%). He is clumsy, with muscle problems, and displays aggressive behavior.

High resolution cytogenetic analysis along with fluorescence in situ hybridization (FISH) for SNRPN and D15S10, revealed an apparently balanced translocation between chromosome 2 and 15 at bands 2q37.2 and 15q11.2. The FISH analysis localized the break between SNRPN and D15S10. More precisely to define the breakpoint in chromosome 15, phage clones from a contig spanning the IPW gene (which lies between SNRPN and D15S10) were utilized for FISH studies. Signals from a 12 kb phage (λ48.34) was detected on both derivative chromosomes, indicating that the break could be localized just proximal to IPW and that no deletion was present. Methylation studies at both DN34 and the SNRPN promoter revealed a normal biparental pattern of methylation.

The use of high resolution chromosome analysis, FISH, and methylation studies of 15q11-15q13 in such an unusual case will aid in the understanding of the cause of Prader-Willi syndrome. Although many translocations associated with the Prader-Willi region have been reported previously, most have involved either a deletion or uniparental disomy. Few cases of apparently balanced translocations in association with the PWS phenotype have been reported. This and other unique translocations are important and can provide insight into: (1) the effects of imprinting in this region, and (2) the search for candidate genes for Prader-Willi and Angelman syndromes, which most likely will include genes distal to SNRPN. Additional studies will include characterization of the phage clone along with more exact breakpoint definition; and analysis of imprinted genes in the PWS/AS region by RT-PCR.

4

MOSAICISM IN PRADER-WILLI SYNDROME. P.A. Mowery-Rushton, J.M. Hanchett, U. Surti. Department of Obstetrics/Gynecology and Genetics, Magee Womens Hospital; Rehabilitation Institute and Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

An accurate diagnosis of Prader-Willi syndrome (PWS) involves a multidisciplinary approach. It is often difficult because the phenotype can be subtle and change dramatically with age. This can be further complicated by the wide variation in phenotypic expression that is found among affected individuals, often leading to an ambiguous diagnosis. Confirmation of the diagnosis can be made by the identification of a deletion of chromosome 15q11-q13. The most effective method for the detection of microdeletions is fluorescent in situ hybridization (FISH) analysis. There are currently four commercially available probes (Oncor), which are located within the classically deleted region on chromosome 15.

We have used FISH analysis to examine a series of 15 PWS patients referred from the Rehabilitation Institute in Pittsburgh for either atypical findings or a deletion not detected with standard cytogenetics. Six of the patients (40%) had a deletion of the 15q11q13 region. Five of the patients (33.3%) did not have a detectable deletion. The most significant finding was the detection of two patients (13.3%) who showed definite mosaicism for a deletion and two other patients (13.3%) who may have low level mosaicism for a much smaller region.

Two of these patients were mosaic for a normal cell line and a cell line with a major deletion. These patients demonstrated a deletion in 32% and 61% of their cells. One case was diagnosed as an atypical PWS who was cytogenetically normal. The second case had typical PWS and displayed all of the classical abnormalities and a deletion was detected cytogenetically.

The remaining two patients appeared to have a mosaic deletion involving only the *D15S10* locus. However, the level of mosaicism was extremely low, 14% and 15%. Both of these patients were classified as atypical. There have not been any other cases reported that were only deleted at the *D15S10* locus. We are attempting to determine whether the low level mosaicism is real or an artifact of the D15S10 hybridization efficiency. We are also analyzing the methylation patterns of the imprinted *SNRPN* gene, which may provide us with some additional information about these unusual cases. It would be interesting to examine other tissues, such as skin biopsy, buccal smears, or surgical specimens in these patients in order to determine whether the level of mosaicism is higher in tissues derived from different germ layers.

The atypical presentations in three of the patients with mosaicism may correspond to the lower level of mosaicism and/or the extent of the deletion. The patient with a deletion in 61% of his cells does not have any atypical anomalies and was diagnosed as typical PWS. This suggests that a threshold level of mosaicism can give rise to the classical phenotype. The significance of low level mosaicism is not known but may lead to misdiagnoses, especially in cases with atypical manifestations. These findings stress the importance of examining a sufficient number of cells for the detection of mosaicism. Currently, there are no guidelines that address the detection of mosaicism using FISH.

5

ADVANCES IN BOTH POSTNATAL AND PRENATAL DIAGNOSIS FOR PRADER-WILLI SYNDROME (PWS). T. Kubota, S.L. Christina, B. Horsthemke, D.H. Ledbetter. Diagnostic Development Branch, National Center for Human Genome Research, National Institutes of Health, Bethesda, MD; Institut für Humangenetik, Universitätsklinikum, Essen, Germany.

Molecular cytogenetic and other molecular methods are currently utilized for diagnosis of Prader-Willi syndrome (PWS). Fluorescence in situ hybridization (FISH) can be utilized to identify the ~70% of cases caused by a deletion of chromosome 15q11-15q13. Molecular methods currently available include methylation analysis of the PW71 locus and microsatellite analysis, which identify both deletions, and maternal uniparental disomy 15 (UPD 15), which together comprise >95% of PWS cases. A new methylation analysis of the SNRPN gene, a candidate gene for PWS, has recently been developed and was used to compare the results of 487 patients suspected of having PWS who had been previously analyzed at the PW71 locus. Also, prenatal diagnosis of UPD 15 has been developed using microsatellite analysis on uncultured amniotic fluid following mosaic trisomy 15 in chorionic villus sampling (CVS).

A parent of origin specific DNA methylation difference was recently detected in the CpG island at the 5′ end of the SNRPN gene, where the maternal chromosome is completely methylated and the paternal chromosome is completely unmethylated. PWS patients with either a deletion, UPD 15, or an imprinting defect show only the maternal, methylated band by Southern analysis following digestion with the methylation sensitive enzyme, NotI. To assess the usefulness of this analysis for routine diagnostics, a study was performed comparing the results of the SNRPN methylation analysis on a cohort of 488 patients whose methylation status at the PW71 locus had been previously assessed. Out of 488 patients, 159 demonstrated only the 4.2 kb maternal band, consistent with PWS, whereas 329 showed

the normal pattern with both the 4.2 kb maternal and the 0.9 kb paternal bands. These results were completely consistent with the previous methylation analysis using PW71. Therefore, the SNRPN methylation analysis provides an additional diagnostic method to detect PWS patients with either a deletion, UPD 15, or an imprinting defect.

Maternal UPD 15 is responsible for ~25% of PWS cases and is associated with advanced maternal age. One mechanism causing UPD 15 involves a maternal meiosis I nondisjunction error producing a trisomic conceptus followed by a random loss of the paternal chromosome 15. Prenatal diagnosis was performed on four cases referred for advanced maternal age with mosaic trisomy 15 in the CVS. In all four cases, follow-up cytogenetic analysis of the amniotic fluid (AF) was normal. Using a modified PCR method to allow rapid molecular analysis on uncultured amniotic fluid, microsatellite analysis revealed one case with maternal UPD 15 and three cases with normal, biparental inheritance. These data are consistent with the hypothesis that random loss of one chromosome in a trisomic conceptus would lead to UPD in one-third of the cases. These results indicated that UPD 15 testing should be considered in women of advanced maternal age with mosaic trisomy 15 in the CVS followed by a normal amniotic fluid analysis to rule out Prader-Willi syndrome.

6

FEW PHENOTYPIC DIFFERENCES BETWEEN PATIENTS WITH PRADER-WILLI SYNDROME DUE TO DELETION 15q AND UNIPARENTAL DISOMY 15. S.B. Cassidy, M. Forsythe, S. Heeger, R. Nicholls, S. Swartz. Department of Genetics, Center for Human Genetics, Case Western Reserve University and University Hospitals of Cleveland, Ohio.

Prader-Willi syndrome (PWS) can result from deletion of the paternally derived 15q11-q13 or from maternal uniparental disomy (UPD) for chromosome 15. Investigations of phenotypic differences between these two groups have not been reported since the era of fluorescence in situ hybridization (FISH) to detect unequivocally the deletion and PCR studies for detection of UPD. Prior to that time, Butler et al. observed that patients with cytogenetic 15q deletions were more likely to be hypopigmented, and there was a suggestion that those without such deletions had a slightly lower mean IQ. In patients with Angelman syndrome, which can be due to maternal deletion 15q11-q13 or to paternal UPD 15, recent studies have suggested that patients with UPD had a milder phenotype. We therefore did a retrospective review of patients with PWS followed by one of us (SBC) to compare manifestations in those with deletion and UPD as determined by molecular testing.

Charts of 54 patients with molecular testing, of which 36 had deletion and 18 had UPD, were reviewed. There were 24 females (16 with deletion, 8 UPD) and 30 males (20 with deletion, 10 UPD). Age range was 2 months to 57 years (deletion: 2 mo–57 yr; mean 16 yrs. UPD: 3.5 yr–40 yr; mean 17.6). Results were as follows:

There was no evidence of difference in frequency of neonatal hypotonia or need for NG tube, behavioral disorder requiring intervention, cryptorchidism, hypoplastic genitalia, hyperphagia, sleep disturbance, viscous saliva, small hands/feet, scoliosis/kyphosis, diabetes, dental abnormalities, age at walking, mean IQ (deletion: 70; UPD: 63), or mean height by sex. The proportion of patients with atypical findings who had UPD was not greater. Several differences between the groups emerged:

	Deletion		Disomy	
Hypopigmentation	16/33	49%	2/13	15%
Typical facies	33/36	92%	10/18	55%
Skin picking	21/27	78%	7/17	41%
Articulation abnorm.	22/23	96%	10/14	71%
High pain threshold	10/10	100%	3/6	50%
Skill with puzzles	13/13	100%	1/4	25%

It is of interest that no patients were identified as having UPD who were under 2 years of age and that the average age of females (22 years) was greater than that of males (13 years), suggesting that females and patients with UPD may have delayed diagnosis.

Although this study is small and retrospective, it suggests that, unlike in Angelman syndrome, PWS due to disomy may not be milder than that due to deletion. In addition to the known difference in frequency of hypopigmentation (explainable by the presence of an albinism gene in the usual deletion), the other major difference between the two groups is that the facial phenotype is less often typical with UPD. In addition, the proportion of patients with some of the subjective characteristics such as skin picking, abnormal articulation, high pain threshold, and skill with puzzles may be greater when deletion is present.

7

A COMPARISON OF PHENOTYPE IN PATIENTS WITH PRADER-WILLI SYNDROME (PWS) RESULTING FROM INTERSTITIAL DELETION AND UNIPARENTAL DISOMY. J. Mitchell, S. Langlois, G. Gillessen-Kaesbach, B. Horsthemke, R. Michaelis, A.A. Schinzel, S. Abelovich, I. Lerer, S. Schuffenhauer, M. Guitart, W.P. Robinson. Department of Medical Genetics, University of British Columbia, Vancouver, Canada; Institut für Humangenetic, Universitätsklinikum, Essen, Germany; Greenwood Genetic Center, Greenwood, SC; Institute of Medizinische Genetik der University Zürich, Switzerland; Hadassah Hebrew University Hospital, Jerusalem, Israel; Kinderpoliklinik der Universität, München, Germany; Hospital de Sabadell, Sabadell, Spain.

Two main mechanisms result in Prader-Willi syndrome (PWS): paternal interstitial deletions of 15q11-q13 and maternal uniparental disomy (UPD). As each mechanism would result in different gene dosage in the proposed critical region, one might expect that PWS resulting from UPD may have a milder phenotype when compared to deletion patients. A number of recent investigations examining uniparental disomic Angelman syndrome patients have supported this hypothesis, but there has been only one study examining this issue in PWS. Another reason to expect a phenotypic difference between the two groups is the possible association of placental trisomy 15 mosaicism with UPD 15. In this case, placental mosaicism may affect birth parameters in UPD patients.

We investigated clinical presentation of 64 PWS patients with maternal UPD and compared them to 49 patients with deletion in the critical region. Results indicated that the length of gavage feeding was significantly less (3.1 vs. 6.8 weeks; $P < .05$) in disomic patients and that the onset of hyperphagia was significantly later (2.6 vs. 1.7 years; $P < .05$) in disomic patients when compared to deletion patients. There were no significant differences for the following criteria: reduced intrauterine movement, birth length and weight, gestational age at birth, neonatal hypotonia, hypogonadism, weight and height on last examination, fair hair relative to parents, indifference to pain, characteristic face, strabismus, and onset of walking.

Our study indicates that there are no major differences between UPD and deletion PWS patients. If UPD 15 is associated with some level of trisomy 15 placental mosaicism, it does not seem to affect birth parameters. Furthermore, there is no major compensation for the lost paternal gene(s) by maternal gene(s) despite that only a subset of genes in the deletion region have been found to be imprinted. However, the fact that onset of hyperphagia was later and dependence of gavage feeding was less in UPD patients may indicate that there is some minor compensation by the extra maternal gene(s) resulting in a slightly milder phenotype. Longitudinal studies are necessary to further elucidate subtle differences between the two groups.

8

TWO UNRELATED FEMALES WITH A PRADER-WILLI-LIKE SYNDROME, HAND CONTRACTURES, NORMAL GONADAL DEVELOPMENT, AND SIMILAR FACIAL FINDINGS. V.A. Holm, N.D. Dinno. Center on Human Development and Disability, University of Washington, Seattle, WA

Persons with Prader-Willi syndrome confirmed by modern laboratory tests (FISH, family DNA studies, and methylation screen) clinically tend to be fairly homogenous. Clinicians familiar with the syndrome often carry clinically atypical patients in their caseload, whose laboratory studies now have turned out to be negative. We are presenting two such patients. They are unrelated teenage females with hand contractures, normal gonadal development, and similar facial features. They might be examples of Urban-Rogers-Meyer syndrome or constitute a new condition.

Patient 1 is an 18-year-old female who has carried a diagnosis of clinical PWS since she was 10 years of age. In spite of satisfying the clinical diagnostic criteria for PWS—9½ points with 5 from the major group, with only 8 points necessary—it has become increasingly evident that this patient's presentation is atypical. She was diagnosed with mild arthrogryposis in infancy because of hand contractures. Her facial anomalies are not typical for the syndrome. Breast development was evident at 13 years and she started to menstruate at age 13½; her periods are heavy and regular. In addition to mild mental retardation, this youngster has a very severe expressive developmental aphasia, which is unusual in the PWS syndrome.

Patient 2 is now 14½ years old. She was inactive in utero and hypotonic at birth. Enlarged nipple openings had to be used for feedings in infancy, dripping the food into her mouth. However, she never had a period of failure-to-thrive and might have been chubby already by 6 weeks of age. When first seen at 9 years of age, she was short, below the 5th centile also for midparental height, and obese with weight for height well above the 95th centile. Her developmental level ranged from 15 to 25 months, placing her in the severe to profound range of mental retardation. She had a some-

what ataxic gait and walked with her arms abducted and hands raised to the shoulder level; she also frequently smiled giving her an appearance similar to that seen in Angelman syndrome. Her head circumference has remained close to −2 SD for age. This girl's developmental progress has continued to be exceedingly slow. Within her limited capability, she sometimes forages for food. Her foot and hand length are below the fifth centile for age, the hands showing a straight ulnar border and a marked ulnar deviation has developed into contractures. She started menstruating at 13 years 4 months of age, shortly followed by breast development. Facial appearance is similar to that of patient 1. Her score on the diagnostic criteria for PWS is 7 with 4 from the major group.

Three Prader-Willi-like conditions with hand contractures have been described in the literature. The disorder depicted by Vasques et al. in 1978 in five male patients in three generations is sex-linked recessive and not pertinent to our patients. The same year Urban et al. published a report describing two brothers with a condition later (in 1983) characterized as Prader-Willi habitus with osteoporosis and hand contractures by Hall. A possible second case of the Urban-Meyer-Rogers syndrome was published in 1988 by Pagnan and Gollop. In 1993, Camera et al. characterized another condition they called "Another Postnatal-Onset Obesity syndrome." Our patients will be compared to the findings in the latter three reports.

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METABOLIC RATE DURING REST AND EXERCISE IN ADULTS WITH PRADER-WILLI SYNDROME. B.L. Bakke, C.C. Draheim, W. Mendoza, R.C. Serfass. Center for Research in Learning, Perception and Cognition, School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis.

Although metabolic rate during rest (RMR) and exercise may both play important roles in the obesity seen in Prader-Willi syndrome (PWS), few studies of these factors have been conducted in people with PWS. Schoeller et al. [Metabolism 37:115–120,1988] reported that resting metabolism was decreased in six adults with PWS when calculated on the basis of body surface area or height, weight, and age, but was normal in relation to fat-free mass (FFM). In contrast, Hill et al. [Dysmorphol Clin Genet 4:27–32, 1990] studied the relationship between RMR and FFM in 36 children and found "patients with PWS had reduced rates in energy expenditure compared to controls, except for patients with the largest body mass and FFM" (p. 27).

Fifteen adults with PWS received extensive training for measurement of RMR and FFM. RMR was assessed after an overnight fast during the last 30 minutes of a 1-hour rest. Expired gases were collected with a face mask and analyzed with a SensorMedics 2900 Metabolic Cart. The mean RMR obtained was 1158 Kcal/day compared with a predicted value of 1521 Kcal/day based on body mass, height, age, and sex. To assess FFM, body density was determined for 13 of the 15 participants on another day using underwater weighing at the residual volume of the lungs. Mean FFM was 37.7 kg; mean weight was 66.5 kg. The mean RMR per kg of FFM was 30.8 Kcal/kg/day.

In their study of metabolism during measured exercise in individuals with PWS, Nelson et al. [JAMA, 233, (6), 627–630, 1973] used a 5-minute step test with seven children and reported a normal cost of physical activity. However, work quantification is more precise on a treadmill or bicycle ergometer [Åstrand and Rodahl, 1977]. We assessed metabolic rate during exercise (EMR) on a treadmill for 13 of the 15 participants using stage 3 of the Modified Balke submaximal exercise test (i.e., a 7% incline at 2 mph). The mean EMR obtained was 960 ml O_2/min. We plan to collect comparison data for RMR and EMR with adults who do not have PWS.

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INFANT DEATH AND CRITICAL ILLNESS IN PRADER-WILLI SYNDROME. T.M. Anaya, C.L. Clericuzio. University of New Mexico Department of Pediatrics, Divisions of Outpatient Pediatrics and Clinical Genetics/Dysmorphology, Albuquerque.

Death of an infant or child under age 5 years with Prader-Willi syndrome (PWS) has been reported only once. In addition, increased risk for severe, life-threatening illness in infancy is not a recognized characteristic of affected individuals. The sudden death of a 9-month-old boy with PWS prompted us to review the stormy first year medical courses of two additional PWS patients. Here, we report on these three patients, including the autopsy findings on the patient who died.

E.S. was a 9-month-old Hispanic boy with typical PWS, found to have a deletion at 15q11-13 by FISH probes. On the day of death he had developed diarrhea and was found unresponsive in the back seat of the family car. Emergency room resuscitation efforts failed and rectal temperature was 103.5°F after 40 minutes. Autopsy the next day showed mild dehydration, acute inflammation of the small bowel, moderate mononuclear meningeal inflammatory reaction, and unilateral anorchia.

B.L. is a 17-year-old Native American woman with typical PWS (albeit with severe cognitive deficit), diagnosed around age 2 years. A deletion at 15q11-13 was demonstrated by FISH probes. Hospitalized frequently in the first 2 years, her most serious illness occurred at age 7 months when she had respiratory arrest and was resuscitated rapidly. She had a fever of 109°F and pneumonia.

R.J. is a 15-month-old Native American girl with typical PWS, diagnosed shortly after birth with 46,XX,del(15)(q11.2q13), confirmed by FISH probes. She has been transported urgently twice to our medical center for life-threatening illnesses. The first was at 11 months for severe dehydration, with fever to 106.8°F and diarrhea. The second was at 14 months for pneumonia. Pediatric critical care was required for both admissions.

Our experience with these three patients suggests that some infants with PWS are medically fragile. A clear cause of death was not determined for the first patient. The only other reported infant death is that of a 6-month-old Japanese female with pneumonia [Hayashi et al., 1992]. Our two Native American patients with critical illnesses have environmental and genetic risk factors, which may increase their risk for infectious diseases, but both had higher fevers and more severe illnesses than are usually seen in Native American infants. We speculate that one reason for a paucity of similar case reports may be that PWS is often not diagnosed in the first year and that infants with unrecognized PWS who die may be categorized as infant death associated with hypotonia/CNS dysfunction. We suggest that increased risk for critical illness be included in the anticipatory guidance for the care of infants with PWS.

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MENSTRUAL PERIODS IN PRADER-WILLI SYNDROME WOMEN. J.M. Hanchett. Rehabilitation Institute, Pittsburgh, Pennsylvania.

The records of 106 female Prader-Willi syndrome patients were reviewed. All had been admitted to the Rehabilitation Institute between 1986 and 1995. The age of menarche, characteristics of menstrual flow, and use of hormones were determined. Ages of patients were 15 to 63. Forty-six patients (43.4%) had spontaneous menarche from ages 7½–38. Most had the onset before age 25. Three patients had premature menarche at ages 7½, 9, and 9½. Most patients had scant, infrequent, irregular menses; several had menorrhagia.

Sixty patients (56.6%) did not have spontaneous menses. Thirteen (12.3%) were given hormones to induce menses. The remaining 47 patients (44.3%) did not have menses, although two-thirds were under 20 years of age and therefore may yet have spontaneous menarche. Many patients required hormone treatment for irregular and/or heavy menses; this was often not successful in regulating menstrual flow. There were no instances of pregnancy.

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THE PREVALENCE AND TYPE OF BEHAVIOR PROBLEMS AND THE CONTRIBUTION OF EXTENDED FAMILY HEALTH HISTORIES AND CURRENT FAMILY STRESS TO SUCH PROBLEMS IN PERSONS WITH PRADER-WILLI SYNDROME: A CROSS-CULTURAL STUDY. B.Y. Whitman. Department of Pediatrics, St. Louis University School of Medicine.

Rational: Behavior difficulties as an age-related component of Prader-Willi syndrome (PWS) is well documented. Common are extraordinary stubbornness, irritability, mood lability, perseverativeness, a tendency toward temper tantrums, rage attacks, and outer-directed aggressiveness. Emotional difficulties in the form of depressions, obsessions, and even frank psychosis are also noted. Yet within the "syndromic norms" of difficult behavior, a wide variation of expression and severity is found. To date, no study has attempted to determine the source of this behavioral variability. This study examines: (1) the prevalence of behavior symptoms and symptom complexes (disorders) in persons with PWS in Australia and the United States, and (2) the possible role of two exogenous sources of behavioral influences: the presence of an extended family history of psychiatric illness, and levels of family conflict and stress.

Methodology: Using a parent informant methodology, a cross-cultural sample consisting of 40 Australasian (Australia and New Zealand) families and 40 families from the United States completed the Survey Diagnostic Instrument (SDI) to detail symptomatology in their child/adult with PWS. The SDI is designed to derive diagnoses for the following DSM-IV categories: neurosis (dysphoric, compulsive, and anxious types), somatization, conduct disorder (violent and antisocial types), and attention/hyperactiv-

ity. In addition, a multigenerational pedigree was constructed to obtain family histories. Family function was assessed with a modified Marital Satisfaction Inventory, modified to remove possible offensive questions regarding sexual functioning. Current family stress was assessed by the Family Inventory of Life Events. The Australasian sample was drawn from parents attending the biannual Australasian PWSA conference in October 1994. The U.S. sample was drawn from parents attending the PWSA-USA conference in July 1994.

Results: This analysis includes 40 different families from each country. The ages of the Australasian PWS sample ranged from 2–35; the U.S. sample ranged from 4–35. Most Australasian patients lived in the family home; the U.S. sample included some persons now in group homes. On preliminary analysis, among the Australasian sample, two males qualify for a diagnosis of attention difficulties, four males qualified for a neurosis-compulsive type diagnosis. Three females scored as mildly depressed and three met criteria of neuroses-compulsive type. Among the U.S. sample, two males qualify for an attentional diagnosis and five met criteria for neuroses-compulsive type. Among females, five met criteria for an attentional disorder, one met criteria for somatization disorder and six met criteria for neuroses-compulsive type. One female met criteria for a psychotic disorder.

Preliminary analysis suggests little association between a family psychiatric history and serious behavioral difficulties in a person with PWS. Interparental conflict or ineffective resolving of differences, both of a global nature, followed by specific child-rearing conflicts and disagreements about discipline appears more contributory to severe behavior difficulties.

Discussion: This preliminary analysis suggests that organically driven symptomatology is primary in this population. The symptom spread suggests diffuse and deviant behavior differences, unrelated to family history. These behaviors appear to be minimized or exacerbated depending on the structure of the family environment. A more complete symptom analysis is necessary to determine those symptoms found in all persons with PWS and those that may be learned behavior. In addition, the results suggest pathways for early intervention in helping parents and caregivers manage the difficult behavioral aspects of the syndrome.

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PHENOMENOLOGY OF OBSESSIVE-COMPULSIVE DISORDER IN PERSONS WITH PRADER-WILLI SYNDROME. E.M. Dykens, J.F. Leckman, S.B. Cassidy. Yale University Child Study Center, New Haven, CT; Case Western Reserve University and Center for Human Genetics, Cleveland, Ohio.

Unremitting food preoccupation is a hallmark of Prader-Willi syndrome (PWS), but the disorder includes repetitive thoughts and behaviors outside the food arena. Persistent skin picking is often seen, as are a host of recurrent, intrusive thoughts and behaviors consistent with obsessive-compulsive disorder (OCD; DSM-IV, 1994). Increased risks of OCD are suggested, and this research evaluates this risk in three ways.

We first identified the range, scope, and severity of nonfood obsessive-compulsive symptoms in 91 subjects with Prader-Willi syndrome, ages 5–47 years (M = 19 years). Prominent symptoms included hoarding, ordering and arranging, concerns with symmetry and exactness, rewriting, excessive grooming, and the need to tell, show, or ask. These symptoms were not correlated with maternal obsessive-compulsive manifestations, but were related to increased familial stress.

Second, we compared these symptoms to well-established clinical criteria for OCD. A full 60% of the sample met criteria for OCD. An additional 25% showed key symptoms of OCD, but did not fully meet diagnostic criteria. Children were just as likely as adults to be classified as having OCD.

Third, the study compared symptoms across a subset of 43 PWS adults matched on age and sex to 43 nonretarded adults with OCD. The Prader-Willi and OCD groups showed comparable levels of symptom severity, including distress and adaptive impairment. Although the PWS and OCD groups had more areas of symptom similarity than differences, the PWS group was more likely to hoard and to need to tell or ask. The OCD group showed more religious obsessions and checking compulsions.

We found, then, remarkably high rates of OCD in both children and adults with PWS. Although further work is needed, these data suggest a possible gene locus for OCD in the region of chromosome 15 involvement in PWS. These data also have treatment implications in PWS. As OCD likely involves a disturbance in serotonin, persons with PWS may similarly show reduced brain serotonin functions. Thus serotonin reuptake inhibitors are likely to be of particular help in reducing these symptoms. Findings also underscore the need for treatment that goes beyond dietary management and that targets relations between family stress and a wide range of compulsive behaviors.

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FOOD AND NONFOOD RELATED DIFFERENTIAL OUTCOMES IN EQUIVALENCE LEARNING BY ADULTS WITH PRADER-WILLI SYNDROME. B. Joseph, J.B. Overmier, T. Thompson. John F. Kennedy Center, Vanderbilt University, Nashville, TN; Department of Psychology, University of Minnesota, Minneapolis.

The effect of food-based reinforcement on learning in Prader-Willi syndrome (PWS) is an unexplored issue in the literature. Many parents and professionals believe that involvement of food in any aspect of a learning situation or in connection with daily task demands interferes with learning and performance, because they believe it leads to so much preoccupation with food that it disrupts attention to the tasks to be learned. In addition, it is argued that learning based on food reinforcement will not generalize to situations not involving food. In light of the limited objective information on this topic, one purpose of the present study was to determine whether food had a disruptive or facilitative effect for people with PWS in a category learning situation.

Seven adults with PWS were taught conceptual categories of five elements each using a computer-presented matching-to-sample task under four different conditions. Reinforcers were presented in one of two ways: nondifferentially, i.e., equally associated with the elements in the potential categories, or differentially, meaning that the elements in a single potential category were exclusively associated with one particular reinforcer. These two teaching procedures were used with both edible and nonedible reinforcers. Transfer tests were conducted without feedback on performance following each of the teaching conditions to assess the effectiveness of the procedures and reinforcers. The results of transfer test performance documented increased transfer for four of five subjects under the nondifferential teaching condition when edible reinforcers were used. For the differential teaching condition, transfer test performance was superior to the nondifferential procedure regardless of whether the reinforcers were edible or nonedible.

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PRADER-WILLI SYNDROME: RE-INVENTING THE WHEEL. H.H. Henson, P.D.K. Lee, W.J. Klish, V.Y. Shepard. Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Parenting a child with Prader-Willi syndrome (PWS) can be difficult. Our clinic experience suggests that unlike other parents of disabled children, many parents of children with PWS seem to resist the idea of mutual support. Many such parents seem to feel that their child with PWS is functioning at a higher level than other children with PWS, and they harbor a fear that their child's behavior will somehow regress or degenerate if there is contact with other children with PWS. Therefore, despite the significant stress involved in raising a child with PWS, many parents are reluctant to interact in a support group setting. Instead, we have observed that parents often try to "mainstream" their child both in and out of the classroom. This coping mechanism inevitably leads to unrealistic expectations and frustration for all involved individuals.

The Prader-Willi Syndrome Clinic at Texas Children's Hospital currently follows ~30 children with PWS. In an effort to determine parental receptiveness to the support group concept, we conducted a four-item questionnaire survey. Results are as follows:

N = 12	% of positive responses
1. There is a need for public education about PWS	100
2. Willingness to work with PWSA local and national chapters	75
3. Unwilling to have their child associate with other children with PWS	50
4. Able to manage their child well on their own	80

Although this survey was not designed as a rigorous scientific investigation, the results tend to confirm our overall impression that whereas parents of children with PWS are often supportive of "crusade" efforts, they may be unwilling to take advantage of a more personalized mutual support environment. In a sense, there seems to be a preference to "re-invent the wheel" with their own child. Our feeling is that this approach may be detrimental to the long-term interests of a child affected with a complex disorder. Additional studies of this phenomenon may help in the design of more effective approach to the treatment of children with PWS.

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PSYCHIATRIC MEDICATIONS USED BY PATIENTS WITH PRADER-WILLI SYNDROME. S. Sulzbacher, R. Gardner, Jr. University of Washington School of Medicine, Seattle; University of Texas Medical Branch, Galveston.

Surveys conducted by the Prader-Willi Syndrome Association (PWSA) suggest that ~50% of adolescents and adults with PWS use psychiatric medications. Drug classes include selective serotonin reuptake inhibitors for depression, irritability, and obsessive-compulsive symptoms; fenfluramine for weight control; mood stabilizers such as carbamazepine and valproic acid for impulsivity and temper outbursts (or for seizures in seizure disorders); stimulants for attention deficit and hyperactivity disorder (ADHD); benzodiazepines and buspirone for anxiety; and neuroleptics for delusions and hallucinations.

Evaluation for using such drugs in mentally retarded/developmentally disabled patients, including those with PWS, involves inspection for syndromes defined in the *Diagnostic and Statistical Manual*, 4th ed. (DSM-IV). Major depression, anxiety disorders, intermittent explosive disorders, ADHD, and delusional disorders have well-defined criteria. Diagnosis in turn suggests specific treatments. If full criteria are not met, clinicians may nevertheless focus upon a specific behavior or behavioral constellation for empirical treatment with unharmful medication. Such symptoms may include anxiety, impulsivity, irritability, depression, and self-injury. Rating forms to quantify actual behaviors help measure success and failure of treatment. Neuroleptics should be avoided unless definitely and specifically indicated.

Factors involving how the drug affects the nervous system determine only part of any drug effect. Other factors include patient, parental, and physician expectancy sets as well as patient rebellion such as when side effects signal active drug use despite double-blind and control conditions. Stressful experience may also outweigh drug effects. Controlled, double-blind studies with PWS patients should use a PWS-specific behavior rating form (PWBRF). Methods are under development at Texas Children's Hospital in Houston in conjunction with investigators from UTMBA (Gardner), UT-Houston, and Baylor medical schools. Anticipated study involves a year-long involvement of 24 patient/families to investigate four drugs in three 4-month phases. In the first two phases, the patient will be his/her own control with a different drug used in each. In the third phase using all four months for a trial, there will be four groups: two drugs used concomitantly, one drug only, the other drug only, and no drug. Not only will a 35-item PWBRF be used (standardized with the help of Seattle conference participants), but also a standardized laboratory measure of aggression and impulsivity, food quantity selected at a limited buffet, other behavior rating scales, and a quantified assessment of skin lesions.

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AN OVERVIEW OF THE VANDERBILT UNIVERSITY PRADER-WILLI SYNDROME PROGRAM PROJECT. T. Thompson, M.G. Butler. John F. Kennedy Center, Departments of Pediatrics, Pathology and Orthopedics, Vanderbilt University, Nashville, TN.

The overall purposes of the program project are (1) to characterize clinical manifestations of people with Prader-Willi syndrome (PWS), as they relate to chromosomal and DNA-identified typologies, (2) to investigate specific behavioral, perceptual, neuroendocrine, metabolic, nutritional, and electrophysiological changes that may distinguish genetic/chromosomal subtypes of PWS, and (3) to test several neurochemical hypotheses concerning mechanisms underlying food motivation in PWS while treating people with PWS neuropharmacologically or with exercise therapy. The program project consists of four projects and four cores.

Fifty subjects with PWS and 50 controls will be recruited over a 5-year period, 40% of whom will be 12–17 years of age and 60% of whom are 18 years and older. The program project will compare findings distinguishing the two major PWS typologies. A battery of psychobehavioral and clinical/laboratory tests will be conducted, including: identification of genetic subtypes of 15q; cognitive and affective (e.g., personality) assessments; ecobehavioral observations in the natural environment; radiological examination of bone (e.g., for osteoporosis) and body composition; HPLC neuroendocrine, amino acid and endogenous opiate assessments; nutritional, energy expenditure, including measures on physical activity, lipid storage and metabolism assessments; food preference studies, relational learning, visual perception, and ophthalmology evaluations. The data from these analyses will be entered into a common data base, making it possible to explore patterns of differences and similarities of the foregoing laboratory and clinical tests of persons falling into the two major genetic subcategories of PWS. The four projects conducted by the investigators focusing on specific sub-issues relevant to the unique features of PWS include:

I. Neurobehaviroal pharmacology of food motivation in PWS—D. Delaney, T. Thompson, M. Ebert, B. Bakke.
II. Nutritional and metabolic characterization of PWS—P. Campbell, M. Carlson, M. Sun.
III. Visual perception in PWS—R. Fox.
IV. Relational learning and food motivation in PWS—T. Thompson, B. Joseph, S. Warren.

The four cores are: administrative, quantitative methods, genetics, and clinical and psychobehavioral.